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Trends in Genetics

Forum

Low-cost CRISPR diagnostics for resource-limited settings

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Next-generation sequencing (NGS) has identified disease hallmarks and catalogued a vast reservoir of genetic information from humans and other species. Precise nucleotide-interrogation properties of clustered regularly interspaced short palindromic repeats (CRISPR) proteins have been harnessed to rapidly identify DNA–RNA signatures for diverse applications, bypassing the cost and turnaround times associated with diagnostic NGS.

Global healthcare expenditure is extremely low in middle and low-income countries (19%) as opposed to high-income countries (81%) [1]. This is coupled with limited resources for testing, tracking, and preventing disease. The success of any healthcare surveillance depends on how fast and how accurately a disease has been diagnosed. In certain scenarios, such as an outbreak of a highly localized pathogenic strain of a microorganism, the development of a new diagnostic test might be more cost-effective than the development of new drugs [2]. Moreover, under low-resource settings, access to special equipment, its maintenance, and trained personnel is limited. Notably, the pattern and type of diseases in developing countries usually differ from that of developed countries. This is due to limited access to healthcare, the extent of community surveillance measures, and the evolutionary variation of pathogens linked to ethnic and geographical features.

The leading causes of death in the developing world are due to pathogenic infections, such as malaria, dengue, tuberculosis (TB), Hepatitis B and C viruses, diarrhea, and tropical diseases. Due to massive NGS-driven programs worldwide, the genetic profiles of most organisms are now being catalogued. However, deep sequencing requires specialized equipment and trained manpower, and is expensive, especially when performed for community surveillance of a large number of individuals. Cost to the consumer is a vital determinant of genetic testing, especially to attract more participants in community screening initiatives where counselling is also associated with testing outcomes (Table 1). The need for high-quality starting material, necessity of pooling and barcoding samples, and extended sample preparation procedures all lead to long turnaround times that limit the use of NGS for routine diagnosis, particularly in countries where healthcare infrastructure is poor. In such places, diagnostic tests that can accurately detect short genetic signatures, circumventing the redundancy of sequencing complete genetic material from samples, can offer a multitude of adaptable and cost-effective applications beyond healthcare decisions (Figure 1). This is where CRISPR-CRISPR-associated proteins (Cas)-based diagnosis (CRISPRDx) systems have been largely successful.

CRISPR systems are components of adaptive immune mechanisms in prokaryotes that assist in cleaving foreign nucleic acids. Different Cas enzymes have been identified in various microorganisms that can target specific sites in both DNA (i.e., Cas 9, Cas12, and Cas14) and RNA (Cas13) [3–5]. Various CRISPRDx platforms make use of either the *trans*-cleavage property of the Cas enzyme or its binding (noncleavage) to nucleotide targets. A variety of novel readouts are possible when using such a system, from colorimetry and fluorescence to simple lateral flow outputs on custom-designed paper strips. Furthermore, CRISPR-Cas systems can identify DNA single nucleotide variants (SNVs), expanding its application to diagnosing disease variants, specific pathogenic signatures, and personalized genetic outcomes.

The development of innovative CRISPR-based diagnostic platforms has seen a tremendous boost during the current coronavirus disease 2019 (COVID-19) pandemic. This situation emphasized the urgency to accelerate the development or repurposing of diagnostics, drugs, and vaccines. The innate response to this situation was to escalate the research component involved in the disease outbreak by addressing the knowledge gaps and research questions, and the urgent generation of scientific information. As a result, the pandemic has witnessed the emergence of various CRISPR-based diagnostic tests [6], showing how versatile these tests are and how they can ramp up testing in resource-limited settings. The importance of innovative and affordable diagnostics is imperative in light of mutations in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) genome, some of which have now been associated with higher disease severity and transmission. NGS-based diagnosis of these variants in the population is costly and time consuming, particularly where the infrastructure to perform such experiments is limited. Owing to the speed, specificity, and low cost of CRISPRDx platforms, we will see in the near future more of such tests for rapid screening, especially for evaluating vaccine efficacy across the globe [7] (Table 1).

The ability to detect specific sequences in DNA–RNA immediately opens a plethora of applications. For developing countries, rapid diagnosis of TB (caused by *Mycobacterium tuberculosis*) and its highly threatening antimicrobial resistance (AMR) forms is paramount to tackle mortality rates associated with the disease. For strains of

Table 1. Examples of genetic tests for selected applications in different sectors, highlighting the cost and timelines associated with sequencing-based platforms

Sector	Disease/signature	Method	Commercialized tests	Cost per run/sample[a]	Time duration[a]
Personalized healthcare	Sickle cell anemia	Sequencing	Sanger Sequencing	US$500	4 h
			Illumina Sequencing	US$1500–2500	10–55 h
			Nanopore Sequencing	US$500–1000	1 min–16 h
	Cancer	NGS	CellMax Life: cancer risk test	US$400	4–5 days
		PCR based	Real-time PCR	US$20	2–3 h
	Food sensitivity and intolerances	NGS	Check My Body Health	US$36	4–5 days
Agricultural biotechnology	Bacterial pathogens	Lateral Flow Assay	Pocket Diagnostics	US$23	10 min
	Bacterial, fungal, and viral diseases	NGS	Sanger Sequencing	US$500	4 h
Infectious diseases	SARS-CoV2	CRISPR	SHERLOCK	US$30.15	1 h
			DETECTR	–	40 min
			FELUDA	US$7	1 h
		PCR based	Real-time PCR	US$20	2-3 h
	TB	CRISPR	CRISPR-MTB assay	–	1.5 h
		NGS	CB NAAT/GeneXpert	US$25	2–3 h
Consumer genetics	Family ancestry	NGS	Family tree DNA	US$79	4–5 days
	Dietary insights		DNAfit+ Vita MOJO	US$190	10–15 days
	Personalized skincare		OmeSkin	US$150	7–10 days

[a]Based on publicly available resources.

multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB, routine treatments might be rendered ineffective, putting the patient at high risk. Low-cost, point-of-care CRISPRDx can augment existing tests, such as nucleic acid amplification tests (NAATs), GeneXpert, and real-time PCR tests, which require costly equipment, reagents, and trained manpower [8].

Other than communicable diseases, CRISPR has also shown the ability to detect tumor-specific biomarkers. Diagnosis of genetic hallmarks associated with cancer using CRISPR could significantly boost early detection, particularly because current screening methods are invasive, lack precision, and are expensive given that specialized sequencing platforms or droplet digital PCR (ddPCR) methods need to be used [9]. Furthermore, researchers have shown that, in advanced cancer stages, patients may acquire mutations in genes, such as estrogen receptor 1 (*ESR1*) and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) [10]. The ability to detect point mutations using CRISPRDx can significantly aid tumor progression studies, leading to improved clinical decisions.

CRISPRDx can be utilized for detecting and diagnosing plant pathogens that reduce yield and have detrimental effects on crop productivity [11]. Agriculture being the economic backbone of several developing nations, the timely diagnosis and intervention of these pathogens can significantly reduce the costs associated with disease management. Similarly, novel diagnostic screening tools that identify signatures of high-yield varieties, especially as an increasing number of genome-edited crops are produced for consumption, will help in tracking and distinguishing them. A similar approach for identifying genetic signatures of indigenous medicinal plants will help in tracing such varieties in the wild, ultimately leading to improved understanding of their cultivation. Overall, these approaches can contribute to a positive economic impact in the agricultural sector.

In the coming years, consumer genomics will become increasingly dominant in developing countries in terms of determining lifestyle decisions. Personal genome sequencing is now routinely used for tracing ancestry, solving paternity disputes, or identifying criminals [12]. The advent of CRISPR-based detection of mutational hotspots or gene signatures can bring the benefits of personalized genetic profiling to countries where the cost and infrastructure associated with whole-genome sequencing is unaffordable.

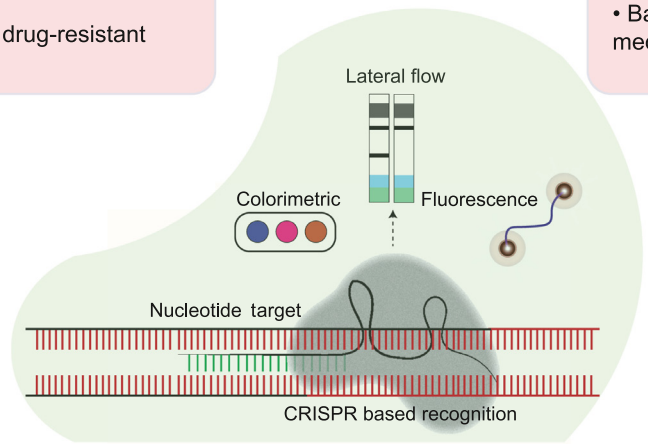
Despite the evolving healthcare system, the world continues to be confronted

Personalized healthcare

- Diagnosis of monogenic/ polygenic mutations in DNA/RNA; pre/post natal genetic diagnosis
- Early detection of cancer biomarkers
- Identifying drug-resistant pathogens

Agricultural biotechnology

- Detection of plant pathogens for early intervention and pesticide resistance
- Selection of high-yield crop varieties
- Barcoding of indigenous medicinal plants



Community surveillance

- Rapid detection of pathogenic microbes during disease outbreaks
- Correlating vaccine and public health interventions with evolving strains of pathogens
- Food/water safety measures, monitoring sanitation

Consumer genomics

- Personalized dietary and culinary choices correlated with genetic signatures
- Clothing, cosmetics and lifestyle products
- Ancestry, paternity, law enforcement

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Figure 1. Applications of CRISPRDx for low cost diagnostics: a snapshot of various domains where NGS based methodologies can be augmented. Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRDx, CRISPR-CRISPR-associated proteins (Cas)-based diagnosis; NGS, Next-generation sequencing.

with prevailing, re-emerging and unknown infectious disease threats. While sequencing-based methodologies for genotyping have become less expensive in recent years due to the advent of pore-based techniques, CRISPRDx brings the advantages of speed, precision, and true point-of-care use: the hallmarks of a good diagnostic tool. As with other evolving innovations, an open and forward-thinking regulatory framework will be critical to its success.

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Declaration of interests

The authors have filed provisional patent applications in relation to certain aspects of CRISPR diagnostics.

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